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Nosocomial Transmission of Hepatitis C Virus in Haemodialysis Patients

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A systematic virological follow-up of 114 haemodialysis patients treated in the same unit showed that 37, including 17 PCR positive patients, were seropositive for hepatitis C virus (HCV). Type 1b HCV was detected in 10 patients and was much more frequent in this population than in the whole population of patients treated in the hepatogastroenterology departments in southeastern France. The E1/E2 genomic region of seven type 1b HCV strains was sequenced. In four patients, a similar strain was detected in both the E1 variable region and the E2 hypervariable region (HVR1). In addition, two of these four patients were seronegative and PCR negative at the beginning of the study and had not been transfused or transplanted during this period. A phylogenetic tree was drawn which confirmed that these strains were very similar and showed that HCV was transmitted via the nosocomial pathway in this haemodialysis unit.

KEY WORDS: hepatitis C virus, genotype, nosocomial infection, E1 region, nucleic acid sequencing

INTRODUCTION

Hepatitis C virus (HCV) infection occurs frequently in haemodialysis patients [Galbraith et al., 1979; Yoshida et al., 1992]. The prevalence of HCV antibodies has been reported to range between 2% and 60% [Esteban et al., 1989; Gilli et al., 1990; Jeffers et al., 1990; Schlipköter et al., 1990; Yameguchi et al., 1990; Almroth et al., 1991; Hayashi et al., 1991; Lin et al., 1991; Jonas et al., 1992; Machida et al., 1992; Hruby et al., 1993; Huang et al., 1993, Vitale et al., 1993; Seelig et al., 1994; Simon et al., 1994]. Numerous factors increase the risk of this infection in haemodialysis patients, including blood transfusion, partial immunodeficiency and frequent parenteral interventions. In 1993, a systematic virological follow-up of HCV infection was initiated in our haemodialysis unit. The first results showed the existence of a high prevalence of patients who were seropositive for HCV as compared with the general population in the region. Interestingly, the patients had a different distribution of viral genotypes from that of the population of patients under treatment in hepatogastroenterology departments in the region.

As described previously by others [Niu et al., 1992; Jadoul et al., 1993; Medin et al., 1993; Oliva et al., 1993; Fabrizi et al., 1994] follow-up enabled the detection of HCV seroconversions in certain haemodialysis patients, in particular in those who had not been transfused or transplanted during follow-up.

These data are consistent with the transmission of HCV via a nosocomial pathway. Following the studies by Yoshida et al. [1993], Allander et al., [1994] and Sampietro et al. [1995], we attempted to verify this hypothesis using genotyping and sequencing methods.

MATERIALS AND METHODS

Population Studied

This study included 114 patients (56 women and 58 men) under regular haemodialysis treatment in the same unit in December 1994. The mean age was 58 (18–89) and the mean duration of haemodialysis treatment was 56 months (1–294). Of these patients 57% had been transfused previously, and 23% had been transplanted previously.

Dialysis Procedure

The patients in the study were dialysed for 4 hours three times a week using Monitral S dialysis machines from Hospal (Lyon, France), and bicarbonate fluids. The dialysers were never reused. All the patients received heparin and always used the same machine. The "universal precautions" were respected as closely as possible, the machines were disinfected using Dialox (CFPO, Paris, France) and the rooms were cleaned between treatments.

Hepatitis C Serology

Serological screening for HCV antibody was carried out using two third-generation ELISA tests (Ortho Diag-

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nostic Systems, Raritan, NJ, and Murex Diagnostics, Dartford, UK). A sample was considered positive if the two ELISAs were positive with ratios of more than 4, or when ELISA positivity was confirmed by an immunoblotting assay: the Murex HCV immunoblot assay or the RIBA-3 test (Chiron Corporation, Emeryville, CA).

Molecular Characterisation of Viral Strains

PCR screening was carried out with the Amplicor HCV test (Roche Diagnostic Systems, Bâle, Switzerland). The genotypes of the HCV strains were determined using the following methods: 1) one-strand direct sequencing of the Amplicor PCR products, using the Perkin Elmer Amplicycle Sequencing kit (Perkin Elmer Cetus, Norwalk, CT), a 5'Fluorescein primer (4CH: ATGGCGTTAGTATGAGTG) [Ravaggi et al., 1992] and the LKB ALF sequencer (Pharmacia Biotech, Uppsala, Sweden); 2) specific hybridisation assays on PCR products using the Innolipa HCV test in the 5'untranslated region (5'UTR) (Innogenetics, Zwijndrecht, Belgium), and the HCV GEN.ETI.K.DEIA test applied to the core region (Sorin Biomedica, Saluggia, Italy); 3) many samples were tested using the Murex Diagnostics anti-HCV serotyping assay (Murex Diagnostics, Dartford, UK) with which the samples were classified (without subtyping) as types 1–4.

In the case of seven 1b strains, two-strand direct sequencing was carried out on a nested PCR product in the E1 region, using the same methods as described above. RNA was extracted from 100 μl of serum using a guanidinium thiocyanate-phenol-chloroform method (RNA Now, Ozyme, Montigny le Bretonneux, France); reverse transcription was carried out with 10 units of AMV reverse transcriptase and random hexanucleotides (both from Boehringer Mannheim, Mannheim, Germany). PCR was carried out under standard conditions with 2.5 units of *Taq* polymerase (AmpliTaq, Perkin Elmer Cetus, Norwalk, CT). Cycling parameters of the primary PCR were as follows: initial denaturation at 90°C for 10 minutes; 35 cycles with denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute and polymerisation at 72°C for 1 minute; final elongation at 72°C for 10 minutes. One microliter of the resulting mixture was used for a 35-cycle nested PCR under comparable amplification conditions. Amplicons were revealed by ethidium bromide staining and UV irradiation after electrophoresis on a 1.2% agarose gel.

The primary PCR primers were HCV31: 5'CTGCAGGCATGCGGGCTBGGRGTGAAGCARTA (reverse) and HCV36: 5'GGTCAYCGCATGGCWTGGGA (sense), giving a 601 bp PCR fragment between nucleotides 1284 and 1884 [Van Doorn et al., 1994]. The nested PCR primers were HCV37: 5'AAGCAATACACYGGRCCACAYAC (reverse) and HCV38b: 5'CGiATGGCiTGGGAiATGATG (sense), giving a 571 bp PCR fragment between nucleotides 1290 and 1860 [Van Doorn et al., 1994]. The 5'Fluorescein sequencing primers were HCV38b and HCV KB: 5'AKGTGCCARCTGCCRTTGGTGTT (reverse, this study). All the oligonucleotides were supplied by Eurogentec (Sereing, Belgium).

Sequences of the E1 region obtained from seven patients were compared with those published previously from 1b strains, with 1b strains from in-patients from Gastroenterology departments, and with one 1b haemodialysis patient treated at another unit in Marseilles. The evolutionary distance between each pair of sequences was calculated using the Jukes and Cantor method [1969]. An unrooted phylogenetic tree was built using the neighbor-joining method [Saitou and Nei, 1987].

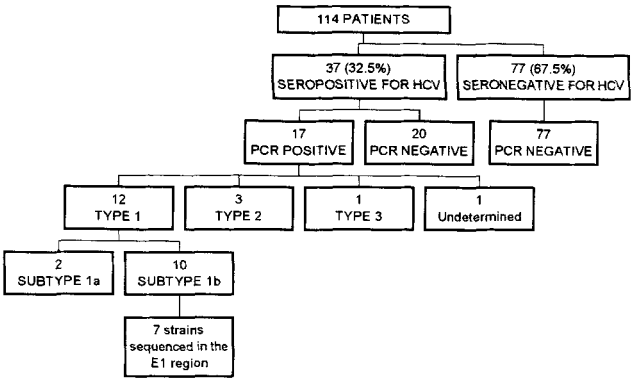


Fig. 1. Results of serological and PCR screening and HCV genotyping.

RESULTS

Among the 114 patients studied, 37 (32.5%) were found positive for HCV serology. Monoreactive "indeterminate" immunoblot was not detected after the serological tests. Seventeen HCV seropositive patients had a positive PCR result for HCV. All of the seronegative patients were PCR negative.

Twelve patients were infected with a type 1 HCV (subtype 1a, 2; subtype 1b, 10), three by a type 2 HCV and one by a type 3 HCV. The type of 1 HCV strain remained undetermined. The results of HCV serological screening, PCR and serotyping are summarised in Figure 1. The results of the genotyping obtained by all four methods used in the study were concordant, as reported in previous studies [Simmonds et al., 1994]. The sequences obtained in the E1 region of the viral genome were compared with those from published previously 1b strains. These sequences are given in Figure 2. Four strains from haemodialysis patients were found to be very similar and formed a distinct cluster in our phylogenetic tree (Fig. 3). Their sequences in the E2/NS1 hypervariable region (HVR1) [Weiner et al., 1991] were determined (Fig. 4) and were found to be very similar.

DISCUSSION

The prevalence of HCV seropositivity in our haemodialysis patients was higher than in the general population (32.5% vs. 1.5% in our region). These patients could have been infected by HCV in various ways. Many had been transfused and it was indeed established that 80% of the seropositive patients had received blood transfusions

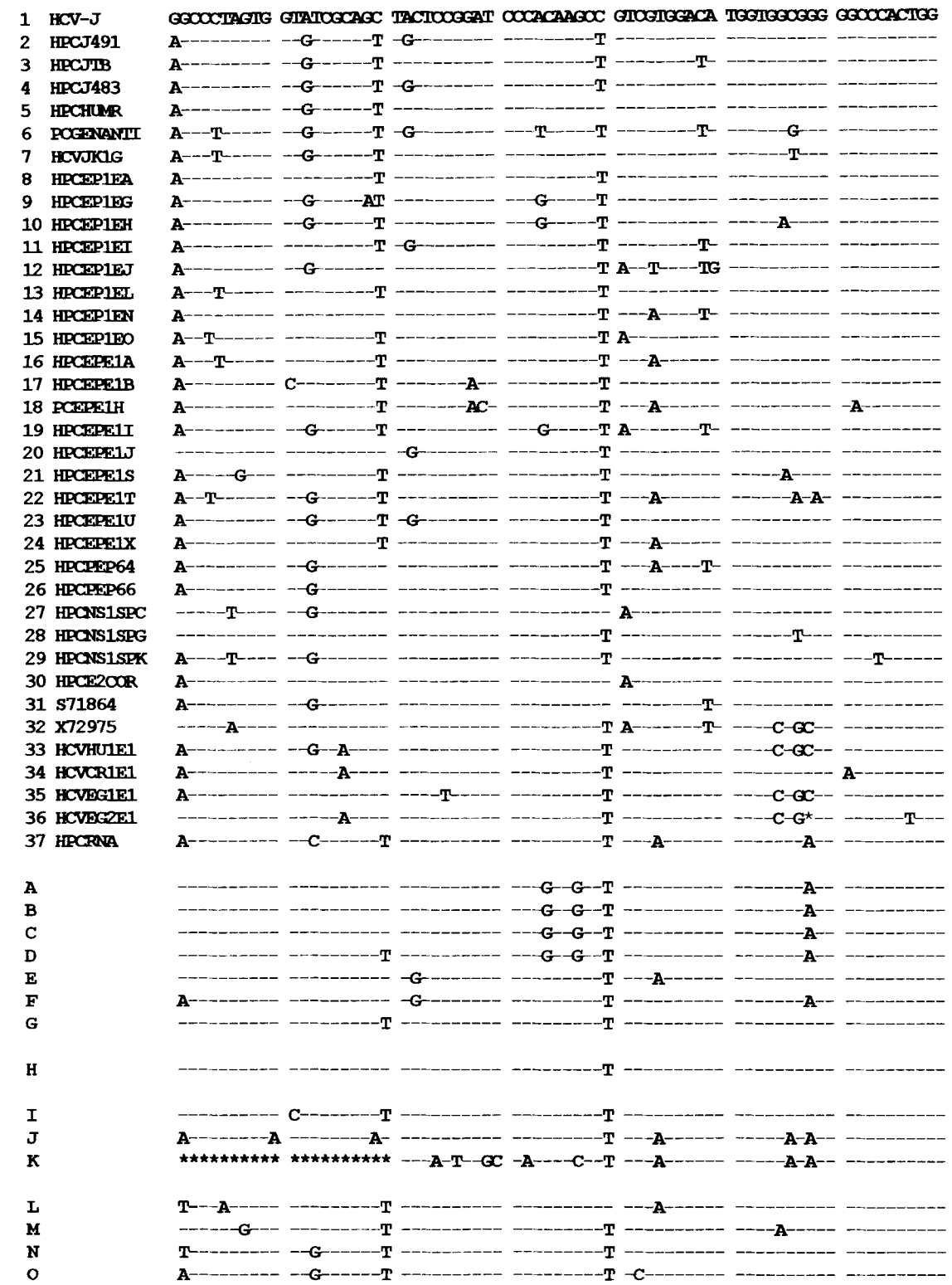
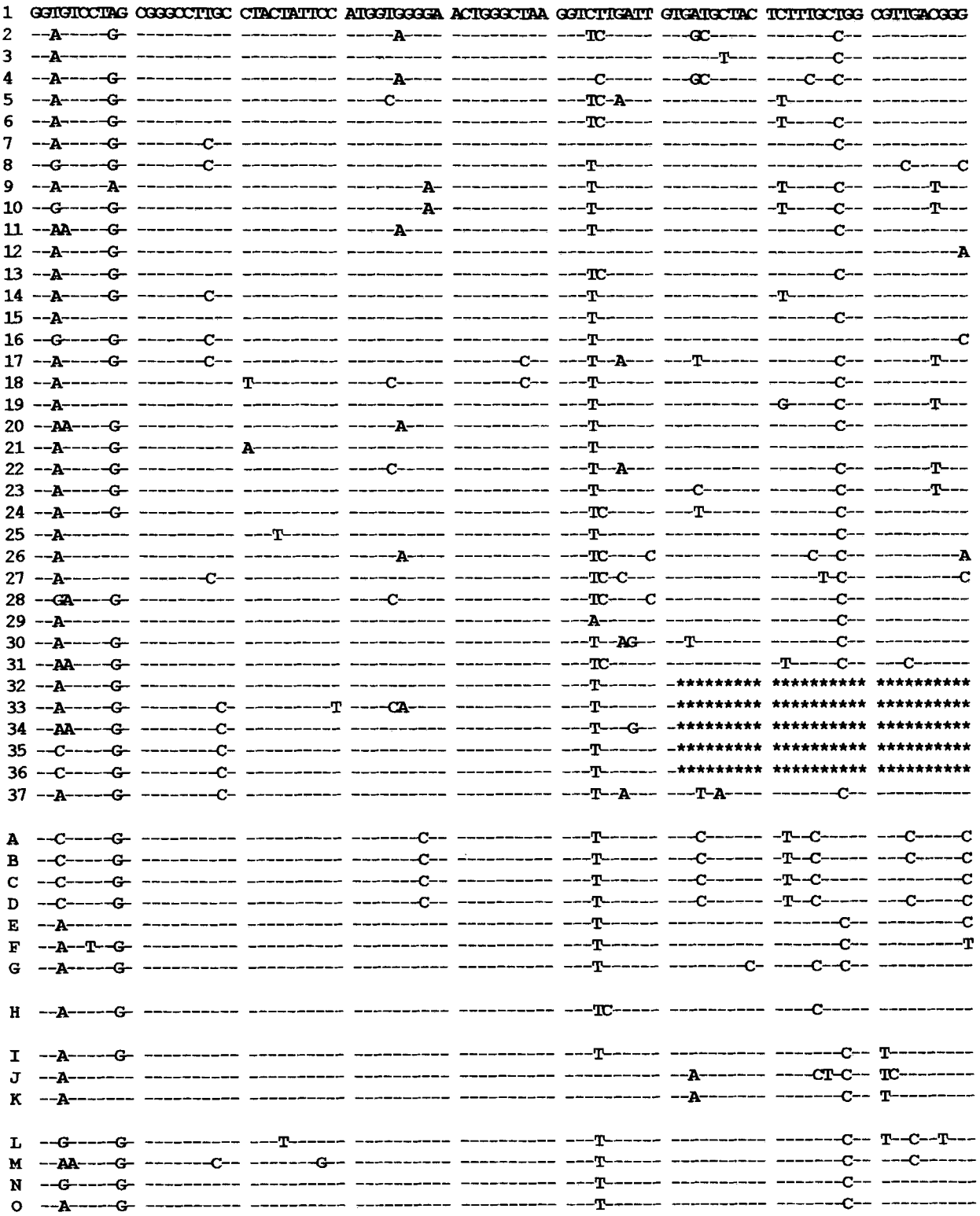


Fig. 2. Sequences in the E1 region of the HCV genome. **1–39:** previously published sequences from 1b strains. **A–G:** sequences of 1b strains from our HD unit. **H:** sequences of one 1b strain from another HD unit in Marseilles. **I–K:** sequences of 1b strains from hepatogastroenterology departments in Marseilles. **L–O:** sequences of 1b strains from hepatogastroenterology departments in Limoges.



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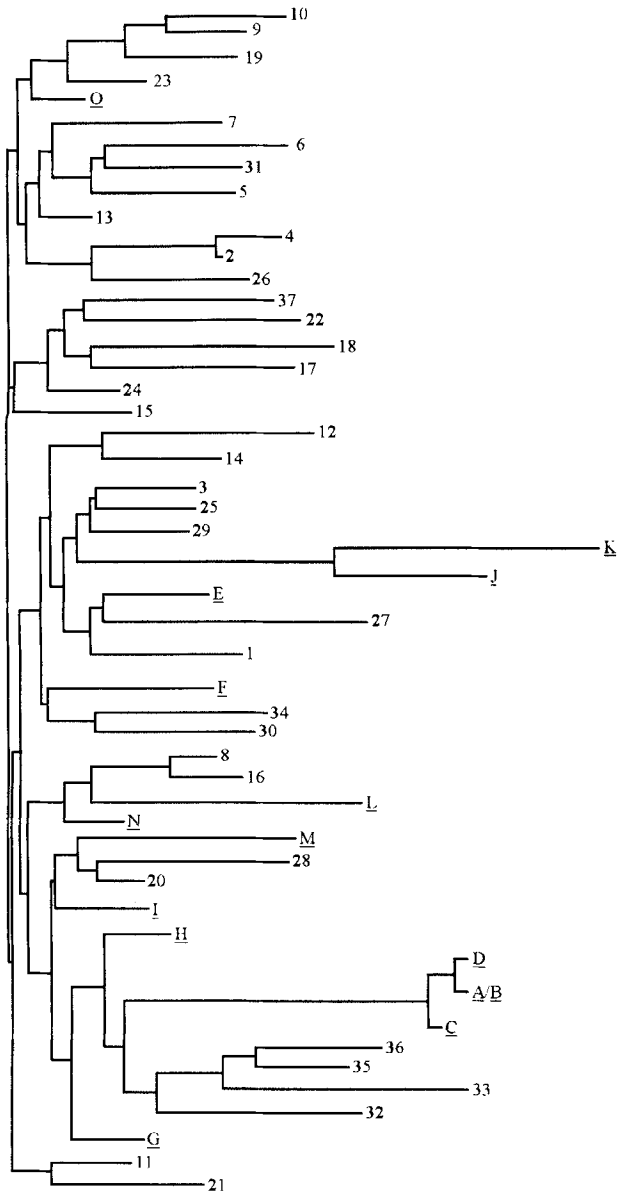

Fig. 3. Unrooted phylogenetic tree from E1 sequence alignments.

vs. only 43% of the seronegative population. The duration of haemodialysis treatment was another important factor since the mean duration was 104 months among the seropositive patients vs. 32 months in the seronegative population. These results are comparable to data published previously [Hardy et al., 1992; Knudsen et al., 1993].

Two facts suggest the existence of alternative pathway of contamination in the haemodialysis unit: 1) the existence of seven seroconversions for HCV in patients who had not been transfused or transplanted during the study period and had been previously found negative for the HCV serology and by PCR; 2) the existence of a higher prevalence of one particular genotype (1b) than in the general population. Ten of the 17 PCR positive samples were type 1b (59%) and only one was type 3 (6%) whereas approximately 40% of all the patients treated for HCV infections in the Hepatogastroenterology departments in the region had type 3 strains, and less than 20% had type 1b strains (unpublished data). This was confirmed using four genotyping protocols based on type-specific sequence characterisation using different technologies (molecular hybridisation and direct sequencing) on two genomic regions (5'UTR and core) and on type-specific anti-NS4 peptide antibody detection.

The HCV strains of seven patients infected with HCV type 1b were identified by sequencing a PCR product in the E1 variable region. These sequences were compared with those previously published from 1b strains, and with sequences obtained from one 1b haemodialysis patient treated in another Marseilles unit and from seven 1b in-patients from Hepatogastroenterology departments (three from Marseilles and four from Limoges, France) (Fig. 2). In four haemodialysis patients (patients A–D), similar sequences were obtained, forming a clearly visible cluster in our phylogenetic tree (Fig. 3). In addition, the sequences in the HVR1 confirmed that these strains were almost identical (Fig. 4).

These data and the fact that two of these four patients became infected by HCV during the study without transfusion or transplantation demonstrate clearly that some of the patients in the unit were infected by nosocomial transmission of the HCV virus. Three of these four patients were regularly treated in the same room but did not share the same machine. The fourth patient had been treated in a different room for at least 2 years. This shows that a systematic study of all HCV seropositive patients is required to clearly establish whether transmission of HCV occurs in a haemodialysis unit.

The study of highly conserved regions, such as the 5'UTR, is not suitable for the exact characterisation of HCV strains. In a previous study, Sampietro et al. [1995] established that the transmission of HCV in a haemodialysis unit was nosocomial, based on the SSCP patterns observed in the 5'UTR, because a rare genotype was responsible for the outbreak. However, this strategy is not suitable for more general use. Like Allander et al. [1994], we decided to study the E1 region because of its variability and we confirmed that the E1 genomic sequences can provide relevant information for making close comparison of viral strains.

The present study was based on a systematic follow-up of haemodialysis patients and was not carried out in response to an outbreak in the department. The viral strains of the patients infected were only sequenced in those who had the predominant genotype. The following results strongly suggest that viral transmission was nosocomial: 1) the high prevalence of infection in comparison with that in the population of patients undergoing peritoneal dialysis [Chan et al., 1991] and with the general population; 2) the fact that some patients had been infected recently; 3) the unusually high frequency of one particular subtype. The sequence determination

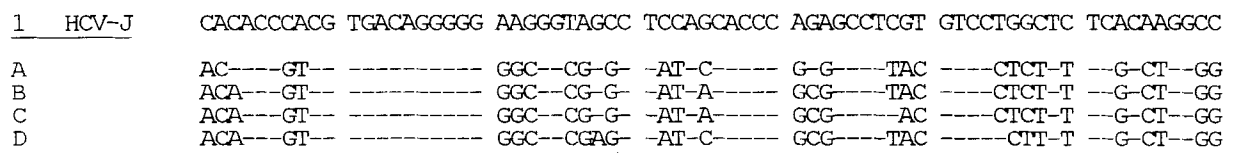

Fig. 4. Sequences in the E2/NS1 hypervariable region (HVR1).

of the genomic regions E1 and HVR1 confirmed this hypothesis.

In conclusion, the results obtained through genotyping and sequencing hepatitis C virus suggest that nosocomial viral transmission occurred in our haemodialysis unit. The exact mechanism involved in the transmission of the hepatitis C virus remains to be discovered and is currently under investigation. To limit the risk of transmission in hospital departments, it is suggested that the same dialysis monitor be used only for HCV positive patients and that an area be set aside for infected patients, with certain nurses in charge of HCV positive patients only. "Universal precautions" should be respected, including the consistent wearing of new gloves whenever the patients are treated and the use of chemical sterilisation procedures after each dialysis session.

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